Brucella species LEVEL A LABORATORY GUIDELINES

Safety

Brucella species are highly infectious! Once this organism is suspected on the basis of clinical and/or laboratory information, do not perform additional testing except within an approved biosafety cabinet and wearing appropriate personal protective equipment!

- 1. Biosafety level 2 practices for specimen processing.
- 2. Biosafety level 3 practices for all activities involving manipulations of cultures.

Colony Characteristics

- 1. Fastidious, usually not visible at 24 hours.
- 2. Grows slowly on most standard laboratory media (e.g. sheep blood, chocolate and trypticase soy agars). Pinpoint, smooth, entire translucent, non-hemolytic at 48h.



Brucella species on sheep blood agar (48 hours)

- 3. Some strains grow on MacConkey agar.
- 4. Grows on Martin-Lewis and Thayer-Martin media.

Microscopic Characteristics

1. Poorly staining, **small gram-negative coccobacillus** (0.5-0.7 x 0.6-1.5 um), seen mostly as single cells and appearing like "fine sand". Bipolar staining is not a distinctive feature. Although a small gram-negative coccobacillus, cells are typically larger than those of *Francisella tularensis*.

Key Characteristics

- 1. <u>Growth</u>: Growth will occur between 20 and 40 C. Grows slowly, but optimally at 35-37 C. CO ₂ enhances growth of some biovars of *B. abortus*.
- 2. <u>Gram stain</u>: Gram-negative coccobacillus
- 3. <u>Catalase</u>: Positive.
- 4. <u>Motility</u>: Non-motile.
- 5. Oxidase: Positive (*B. canis* is variable).
- 6. <u>Urease</u>: Positive. Strong, typically <2h, some within 5 minutes.
- 7. <u>X and V factors</u>: Neither is required.

If a biosafety cabinet is available for testing, oxidase and urease testing may be very useful in differentiating *Brucella* species from the phenotypically similar *F. tularensis*, *Yersinia pestis*, *Haemophilus*, *Bordetella*, *Acinetobacter*, *Pasteurella*, *Moraxella* and *Oligella* (*Moraxella urethralis*)

Isolates with the above characteristics should be reported to the patient's physician forwarded to the South Dakota State Public Health Laboratory for additional testing.

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